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Phylogenetic tree constructed of *Salmonella enterica* subspecies *enterica* isolated from animals and humans in Basrah and Baghdad governorates, Iraq

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Abstract

The genetic relatedness of *Salmonella enterica sub sp. enterica* isolated from human and animal origin has more interest as its data possibly will offer an essential confirmation for the source of human infection. This study aimed to determine the genetic relationship of *S. enterica subspecies enterica* isolated from human and animal sources. A total of 300 samples were collected from two primary sources, human and animal, from two different regions, Baghdad and Basrah governorates. For constructing the phylogenetic Tree of *Salmonella enterica* subspecies *enterica*, the sequencing of PCR product (positive samples) for each target genes *16s rRNA*, *avrA*, and *spvC* were analysed using BLAST analysis to determine the similarities and differences between the Iraqi strains and the existing global strains. The similarity rate in the first gene 97.77%, the second gene 98.29%, and the third 96.82%, respectively. The genetic Tree of each of the three genes was set up separately using two methods, Maximum Likelihood, and the second Minimum evolution. The phylogenetic analysis reveals that Iraqi strains of Salmonella are highly similar, and they share the same sequence of *16s rRNA* gene with national Salmonella strains. However, their bases of *avr*A and *spvC* genes are not similar. This difference leads us to conclude that the Iraqi Salmonella evolution path was characterized by its path in developing global strains with some correlation in some samples; it may be linked with the same ancestors from which it emerged.

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Introduction

Salmonellosis is considered one of the most important zoonotic diseases (1-3). *Salmonella* is most likely to inhabit the abdominal lumen of wide-ranging vertebrates (4-6). Over 2,610 different serovars are recognized by a schematic pattern of Kaufmann-White-Le minor. *Salmonella Enteritidis* and *Salmonella typhimurium* are the worldwide distribution and the most common serotypes that cause Salmonellosis in animals and humans, as they are generally transmitted by infested animals and their productions to humans been (7-10). The investigated genes comprised *16s rRNA*, *avrA*, *and spvC*, in which *avrA* genes that are heading *Salmonella* for inducing inflammation through inhibition of both pro-inflammatory and the antiapoptotic (NF Kappa B) pathway and as a genetic marker for the existence of SPIs, which is related with the increase invasion and intracellular persist in the phagocytic and non-phagocytic cells (11). While the other gene, *Salmonella* plasmid virulence gene C (*spvC*), this gene exists in a virulence plasmid; generally formed by infected of definite host and it always requires for complete expresses of

virulence in *Salmonella* (12), Moreover to promote the rapid growing and surveillance inside the same host. However, all these virulence determinants are broadly spreading among animals, humans, and the environment, with specific variation (13). Alternatively, the *16S rRNA* gene analysis is the most accepting factor for molecular study (14). Genomes of bacterial strain seemed to contain several genes with a specific role. For instance, in bacteria, the *16S rRNA* is recognized to have nine hypervariable areas (V1-V9), which are often used to identify the microorganisms at species level as they show a sequencing diversity in this region many species of bacteria.

Furthermore, many species of bacterial strains have a conserved region closest to these hypervariable parts, and this conserved region considers the quorum sensing sequences that could be amplified by using a universal primer (15,16). The hypervariable area of *16S rRNA* sequences was used in many genetic studies to identify a particular bacterial species or recognize among a different bacterial species (17-19). Additionally, many universal primers, such as the primer used to amplify the variable regions 1,3, and 6 (V1, V3, V6), were previously mentioned in previous studies are wide-ranging based primers that were used to distinguish among the various species of Salmonella (20-22).

Phylogenetic trees were used almost limiting to describe relationships within the species in systematics and taxonomy before the beginning of DNA sequencing technologies. In addition to explaining relationships between species on the Tree of life, phylogenies represent relationships between paralogous in a gene family (23); to understand pathogens' development and epidemiological activity (24,25). More recently, molecular phylogenetics has become an indispensable tool for genome comparisons. It is used to identify genes and classify metagenomic sequences (26,27). Also, to interpret modern and ancient individual genomes and reconstruct ancestral genomes (28-30). So, this study was achieved to determine the genetic relationship of *S. enterica* sub sp. *enterica* isolated from human and animal sources and to determine the similarities and differences between the Iraqi strains and the existing global strains.

Materials and methods

Ethical approve

Ethical approve was taken from Research Ethical Committee of College (RECC). No:6,7/35/1910, Date:21/5/2017.

Samples collection and processing

A total of 300 samples were collected from two regions, Basrah and Baghdad governorates, involving the following samples: 50 stool samples were collected from healthy workers in the field who were in contact with the domestic animals, and another 50 samples were collected from the diarrheic patients. While the animal samples were involved, 50 cows' fecal samples were taken directly from the bowel. Another 50 samples were taken from the rectum of the chickens directly. A total of 50 fresh eggs were gathered from a typical local house of chicken and included two forms of samples: The first was swab samples directly collected after taking out the shell of the eggs. At the same time, the second type of samples was included, taken the contents of whole eggs and directly put in a sterilized container. The final type of samples was involved 50 swab samples were accumulated from sewerage water and the ground area of domestic animals' houses and slaughter tools. According to a previous study, all methods and processing samples collection were carried out (31).

Primary detection of Salmonella

The direct detection of *Salmonella* was mentioned in the previous study (31,32). Further confirmation was done by Polymerase Chain Reaction (PCR) and analysis of DNA sequencing.

DNA extraction

Bacterial DNA from suspected colonies was extracted by suspending colonies of bacteria growth in 200 µl of sterilized DWDW and then boiling at 100 °C for 15 min to lyse the bacteria. After heating, about 800 µl of Sterile DW was added to get 1ml and vortex until the solution was remixed. The purified DNA was then harvested by centrifuge at 12,000 rpm for 10min.

PCR technique

Three sets of oligonucleotide primers, *16s rRNA*, *avrA*, and *spvC*, were used in PCR to detect *Salmonella* species and construct the phylogenetic tree. Oligonucleotide sequences of primers and amplification region are shown in table 1. While the reaction mixture was prepared in a total volume of 25µl for all genes as a following: one µl (10pmol/µl) of forward and reverse primer were added to 5 µl of master mix, then 10 µl of extracted DNA was added as a template the final volume was adjusted by adding 8 µl of Nuclease free water PCR was performed on a PCR thermocycler under the conditions: 95°C for 5min, 94°C for 10 sec, and for annealing (55°C for 30 sec for 16s rRNA gene and 58°C for *avrA* and *spvC* genes), then 72°C (60 sec) for elongation. Cycles were repeated 35×. And the final extension 72 °C for 10 min. The PCR products were investigated by electrophoresis using 1 % agarose gel and staining with ethidium bromide the observed after under UV light (33).

Table 1: The Oligonucleotides Primers Details used under study

Primer name	Sequence 5' 3′	Amplicon size (bp)	Reference
16s rRNA	TGTTGTGGTTAATAACCGCA CACAAATCCATCTCTGGA	572	31
avrA	CCT GTATTG TTG AGC GTCTGG AGA AGAGCT TCG TTGAAT GTCC	422	12
spvC	ACC AGAGACATT GCCTTCC TTC TGA TCG CCG CTATTCG	467	

DNA sequencing, phylogenetic data analysis, and tree drawing

The 20 µl of PCR product (positive samples) with 2 µl for each sample of 17 pica Mole of the F primer for each target genes 16s r RNA, *avrA*, and *spvC* were sent to MACROGEN "http://dna.macrogen.com" for getting the sequencing of the target genes. The crude sequencing is then analyzed using the Basic Local Alignment search tool (BLAST) of the Geneious software to search for a similar sequence previously recorded in the National Centre for Biotechnology Information database (NCBI) htpp:/ /blast.ncbi.nlm.nih.gov. The phylogenetic tree was constructed using the Maximum Likelihood and Minimum Evolution method by MEGA 7 software (34).

Results

The suspected samples from primary identification were analyzed for the presence of *16s rRNA*, *avrA*, and *spvC* genes using three pairs of primers for the amplification of 572 bp; 422bp and 467bp DNA fragments, respectively. PCR results of *16s rRNA*, *avrA*, and *spvC* genes demonstrated that 39 of investigated samples showed this gene's presence (Figures 1-3).

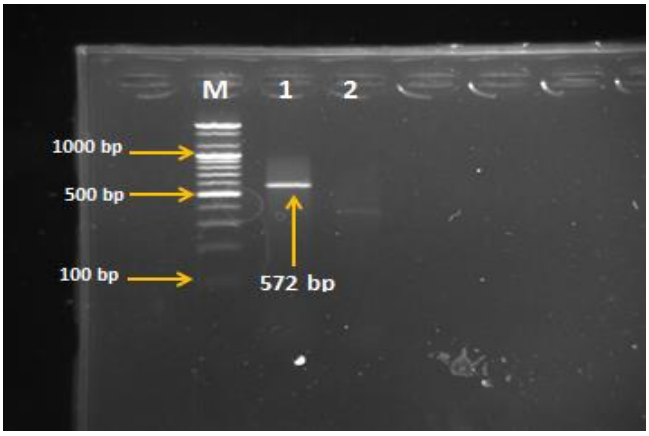

Figure 1: Conventional PCR amplification of *16rRNA* gene (A) for *salmonella* strains. Lane M: molecular marker (100bp), lane one positive result for *16rRNA* gene (572bp), lane 2 for control negative.

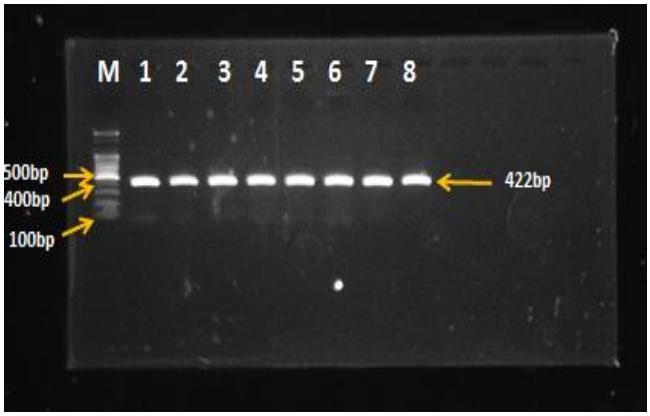

Figure 2: Conventional PCR amplification of *avrA* gene (B) for *salmonella* strains. Lane M: molecular marker (100bp), lane 1to eight positive results for *avrA* gene (422bp).

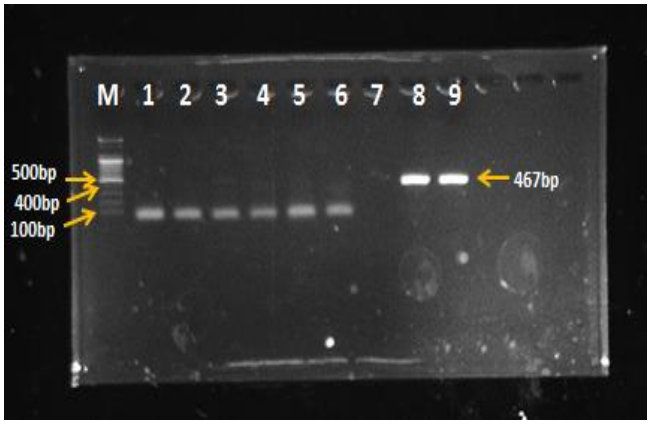

Figure 3: Conventional PCR amplification of *spvC* gene (C) for *salmonella* strains. Lane M: molecular marker (100bp), lane 1 to 6 negative result, lane 8and nine positive results for *spvC* gene (467bp), lane 7 for control negative.

Sequence Analysis and Phylogenetic tree construction

For a complete detection for *Salmonella* spp, the alignment information for each gene was analyzed to see the distribution of the similarity with NCBI information as shown in table 2, which gives the nearest national strains to the Iraqi strains and its identity level. Additionally, the

phylogenetic tree was constructed, and all positions containing gaps and missing data were eliminated automatically by MEGA 7 software. Two methods of phylogenetic tree were used. The first method was (Maximum Likelihood), and the second method was (Minimum Evolution) (Figures 4-6). For recognizing between the three genes, it was written in one latter (A, B, and C) for *16s rRNA*, *avrA*, and *spvC* respectively, and to recognize between Iraqi strains and national strains from NCBI information, a pink color sign was put before national sample with the recording code and the country where it was isolated. On the other hand, 10 of the local Iraqi strains were registries in NCBI, and the accession number was the following: MK968117, MK968116, MK972334, MK940757, MK940799, MK941605, MK920189, MK920200, MK940815, MK940590.

Table 2: Level of Identity for the Iraqi strains with GenBank and giving the accession number for the nearest national strains

Sample No.	Species	Identity (%)	Accession Number
1	*Salmonella enterica sub sp. enterica*	97	LR590082.1
2	*Salmonella enterica* sub sp. *enterica*	97	CP034230.1
3	*Salmonella enterica* sub sp. *enterica*	99	CP034233.1
4	*Salmonella enterica* sub sp. *enterica*	98	MK215845.1
6	*Salmonella enterica* sub sp. *enterica*	99	CP029852.1
7	*Salmonella enterica* sub sp. *enterica*	99	CP029914.1
8	*Salmonella enterica* sub sp. *enterica*	99	CP029900.1
11	*Salmonella enterica* sub sp. *enterica*	95	AF057363.1
14	*Salmonella enterica* sub sp. *Enterica*	98	CP007323.2
21	*Salmonella enterica* sub sp. *Enterica*	99	KJ672306.1
22	*Salmonella enterica* sub sp. *enterica*	99	KU 641446.1
23	*Salmonella enterica* sub sp. *enterica*	99	MH352206.1
31	*Salmonella enterica* sub sp. *enterica*	99	MK972334.1
32	*Salmonella enterica* sub sp. *enterica*	99	CP007323.2
33	*Salmonella enterica* sub sp. *Enterica*	98	CP007344.2
34	*Salmonella enterica* sub sp. *Enterica*	99	MK941605.1
35	*Salmonella enterica* sub sp. *enterica*	98	CP014358.1
36	*Salmonella enterica* sub sp. *enterica*	100	CP014536.1
37	*Salmonella enterica* sub sp. *Enterica*	98	CP007354.2
38	*Salmonella enterica* sub sp. *Enterica*	98	CP007381.2
39	*Salmonella enterica* sub sp. *enterica*	86	CP007377.2
40	*Salmonella enterica* sub sp. *enterica*	98	CP014358.1
41	*Salmonella enterica* sub sp. *enterica*	98	CP007354.2
42	*Salmonella enterica* sub sp. *enterica*	99	CP007309.2
43	*Salmonella enterica* sub sp. *enterica*	97	CP007423.2
51	*Salmonella enterica*	98	LR134233.1
52	*Salmonella enterica* sub sp. *enterica*	97	KT036673.1
53	*Salmonella enterica*	99	MK920189.1
54	*Salmonella enterica* sub sp. *enterica*	98	CP029958.1
55	*Salmonella enterica* sub sp. *enterica*	98	MK215094.1
56	*Salmonella enterica* sub sp. *enterica*	99	CP029960.1
57	*Salmonella enterica* sub sp. *enterica*	99	LN999997.1
58	*Salmonella enterica* sub sp. *enterica*	99	CP029952.1
60	*Salmonella enterica* sub sp. *enterica*	97	KY766065.1
61	*Salmonella enterica* sub sp. *enterica*	98	KX146473.1

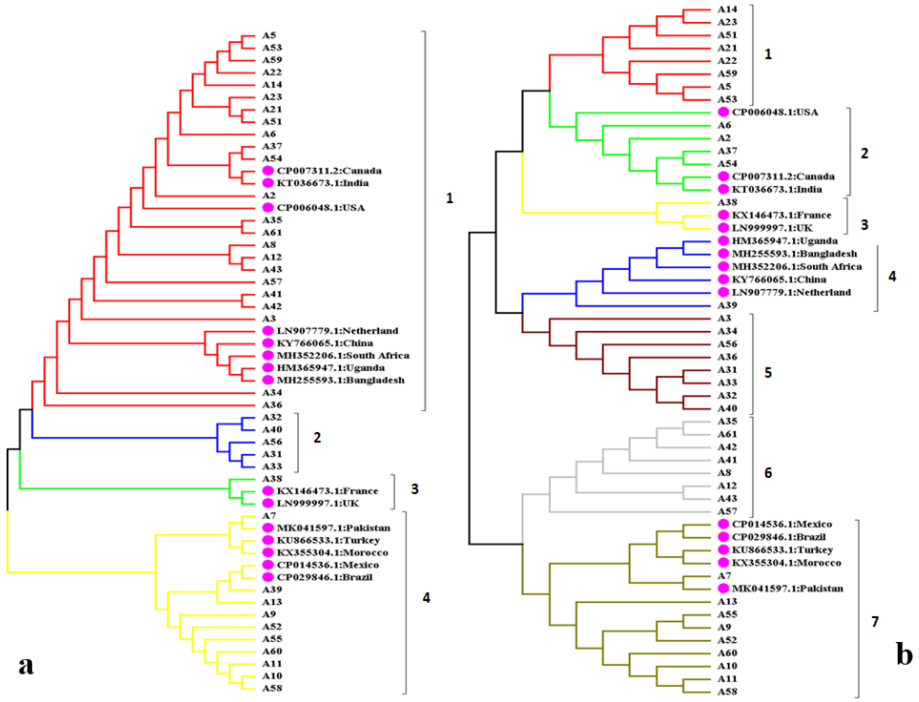

Figure 4: Molecular Phylogenetic Tree of *Salmonella enterica sub sp. enterica* based on 16s r RNA gene A, illustration by using Maximum Likelihood method (a) and Minimum evolution method (b), in which Iraqi strains have the following number: (1-10) Human; (11-20) Chicken;(21-30) Cows; (31-50) Egg and (51-70) other samples.

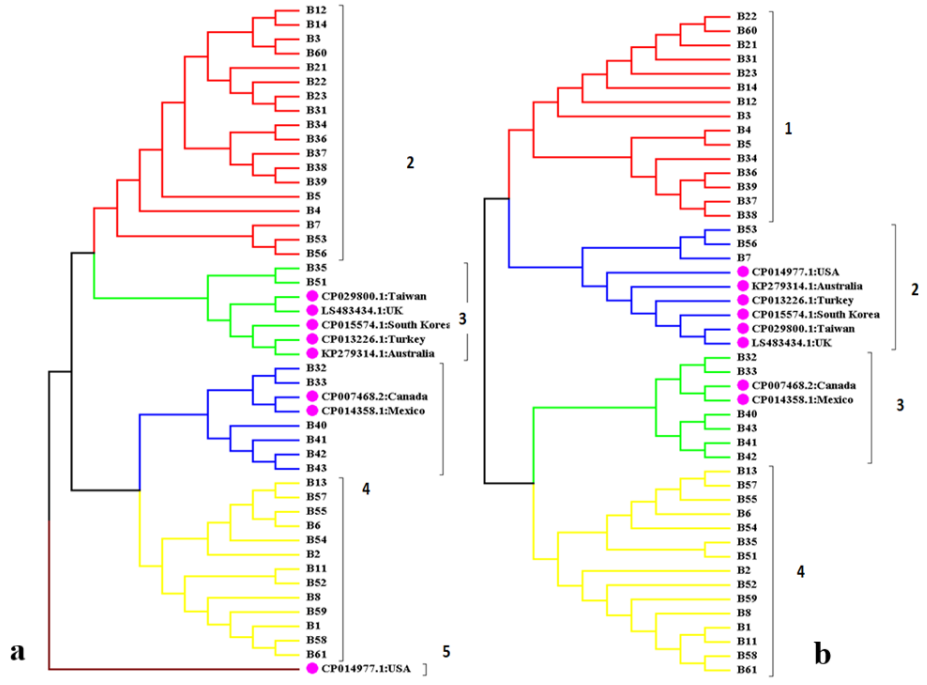

Figure 5: Molecular Phylogenetic Tree of *Salmonella enterica* sub sp. *enterica* based on *avrA* gene (B), illustration by using Maximum Likelihood method (a) and Minimum evolution method (b), in which Iraqi strains have the following number: (1-10) Human; (11-20) Chicken;(21-30) Cows; (31-50) Egg and (51-70) other samples.

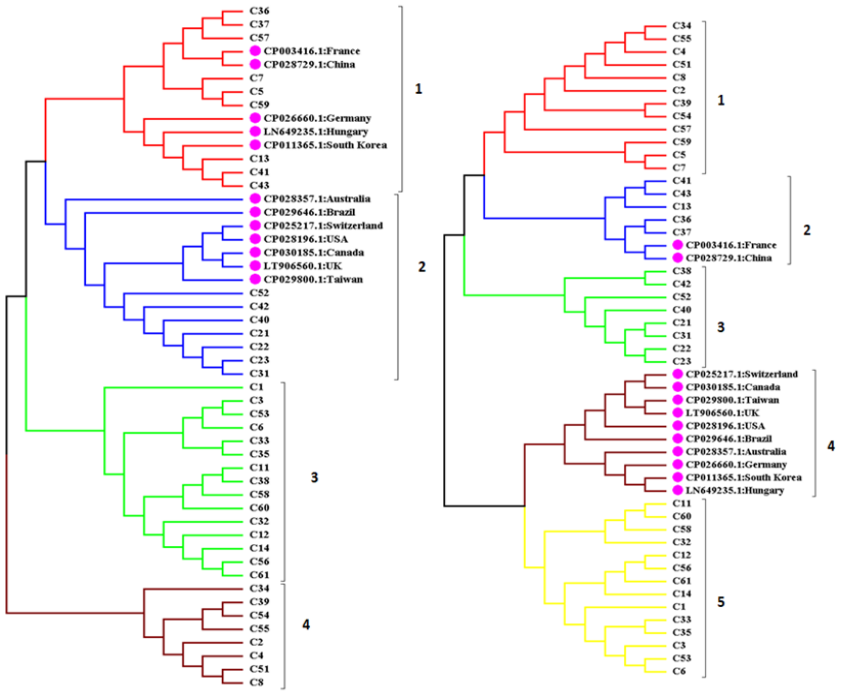

Figure 6: Molecular Phylogenetic Tree of *Salmonella enterica* sub sp. *enterica* based on *spvC* gene (C), illustration by using Maximum Likelihood method (a) and Minimum evolution method (b), in which Iraqi strains have the following number: (1-10) Human; (11-20) Chicken;(21-30) Cows; (31-50) Egg and (51-70) other samples.

Discussion

Salmonella enterica sub sp. *enterica* shows a significant challenge in public health as the total number of cases each year increases due to its ability to spread from animals to humans or by a food chain. A molecular technique that uses the highly conserved sequences such as housekeeping and virulence gene will offer an excellent and multipurpose approach for detecting *Salmonella enterica* sub sp. *enterica*. The molecular technique also helps to correctly identify the sources and routes of transmission of infection, besides the detection of the bacterial population. Additionally, since *S. enterica* sub sp. *enterica* has numerous sub ancestries and shows a complex molecular pathogenicity pathway, understanding the evolution pathway of *S. enterica* is more critical in detecting its sources routes of spreading besides predicting and preventing the occurrences (35,36). In our study, the positive strains of PCR products were sent to MACROGEN, Korea, to get the sequence results and construct the phylogenetic Tree of Salmonella *enterica*. Each sequence was BLAST in the NCBI to compare it with the national sequences of *Salmonella* from different sources and different sites. The comparison of strains was made with 100 national isolates to increase the validity and reliability of this study to find out the site of changes in the sequence to determine if there is a mutation or not between Iraqi strains and national reference. The acquired sequences to the BLAST analysis were done by Geneious software to identity between Iraqi *Salmonella* in this study and regional and national *Salmonella* from GenBank. It was done in two steps, the first one for each gene alone to see the distribution of Identity of this gene with GenBank to know the level of identity of all Iraqi strains for the *16s rRNA*, *avrA*, and *spvC* genes with national GenBank. The second one was done by comparing the three genes together to see if there are relationships in the level of identity of the three genes for the same strains to know the different strains if it is unique or there is an error in the isolation technique. The phylogenetic tree is essential to find the organism's origin and a path of evolution, sequence similarity, and relationship (37). The phylogenetic tree for each gene was made by the maximum likelihood method and minimum evolution method to see the relationship of Iraqi isolates with the higher query cover above 90% of national isolates.

The distribution of the national strains among the Iraqi strains in the group 1 was content Canada, India, USA, Netherland, China, South Africa, Uganda, and Bangladesh and maximum similar of Iraqi strains, while group 3 content France and UK and similar Iraqi strains and group 4 content Pakistan, Turkey, Morocco, Mexico and Brazil with maximum similar Iraqi strains, except group 2 which missed national strains but it shares the same ancestor of

the group 1 which was a branch of the same ancestor of the group 3. On the other hand, the minimum evolution for *16s rRNA* genes like in the maximum likelihood method the distribution of the Iraqi strains and national strains in the most groups of the tree found in the USA, Canada, and India in the second group and France and UK in the third group and Uganda, Bangladesh, South Africa, China and Netherland in 4 and Mexico, Brazil, Turkey, Morocco, and Pakistan in group 7. In contrast, groups 1,5, and 6 arise from the same ancestor of groups 2, 4, and 7, respectively, but that had its evolution branch.

This means the Iraqi strains of *Salmonella* are highly similar to the national *salmonella* strains and share the same sequence of *16s rRNA* gene because this gene is fixed and have a low mutation rate and need a very long time to change, so it is used in the classification of the bacteria in the world. Detection based on *16s rRNA* is a reference method for bacterial identification and taxonomic studies (38).

Moreover, the distribution of Iraqi strains, in strains B35 and B51 were significantly like to the national strains from Taiwan, UK, South Korea, Turkey, and Australia in group 2, and the strains B32, B33, B40, B41, B42 and B43 which were isolated from eggs was maximum similar to the strains from Canada and Mexico in group 3. In contrast, the other groups lacked the similarity to the national strains, and group 5 was unique with USA strains alone which is far away from Iraqi and other strains in this tree. Although the groups 1 and 2 come from the same ancestor and 3 and 4 share the same ancestor, it has a particular branch. Additionally, the evolution in the *avrA* gene, which is divided into two branches each one takes two directions of evolution one similar to national strains as in group 2 content the USA, Australia, Turkey, South Korea, Taiwan and UK and group 3 content Canada and Mexico, while the other direction was unique in evolution as in groups 1 and 4. The mutation effectiveness can explain this in *avrA* gene that led to some change in the Iraqi strains and plays an influential role in the distribution of the Iraqi strains in the tree led to a collection of the national isolates with Iraqi strains maximum likelihood in group 1, which contains France, China, Germany, Hungary, and South Korea and group 2 contains Australia, Brazil, Switzerland, USA, Canada, UK, and Taiwan. In contrast, groups 3 and 4 were lack of similarity to the national strains. Also, only group 2 shows the same evolution similarity with national strains which content France and China. In contrast, other groups had their evolution branch, except group 4, which contains only national isolates Switzerland, Canada, Taiwan, UK, USA, Brazil, Australia, Germany, South Korea, and Hungary and lacks any sharing with Iraqi strains, that may be explained as Iraqi strains had its evolution in *spvC* gene.

All above results were attributed to the evolutionary relationships between the ancestor and new generation in the tree is trunks ancestor and branches or new generation placed at the end of branches that have arisen from the ancestor. The distance between two groups is the indicator for the relationship degree; close branches mean closely related groups. Branch length as long as mean the number of changes (39).

Subsequently, the relationships between the differences and similarities of Iraqi strains and national strains maybe refer to mutation rate due to the environmental conditions, drugs use, and the transportation of bacteria by the animals, foods, and water pollution of rivers. Moreover, attributed to widespread use with over-the-counter accessibility and unrestricted obtaining to common antimicrobials drugs for human and animal usages, these factors create resistant strains (40).

In many countries, the infection rate by *Salmonella* is significantly increased among populations living in the waste water-spreading grounds than those living in the control areas that do not practice wastewater spreading. Furthermore, as a result of persistent occurrence of *Salmonella* in higher concentrations in effluent water and survival for an extended period in the moist soil, in which the cattle feeding on drain water irrigated pasture leads to be infected with Salmonella. Additionally, people may have the bacteria when consuming milk or ingestion meat from such infected cattle (41).

Conclusions

We concluded that Iraqi strains of *Salmonella* are highly similar to the national Salmonella strains and share the same sequence of *16s rRNA* gene, but show some dissimilarity on the bases of *avrA* and *spvC* genes, which could be attributed to the mutation.

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Conflict of interest

Conflict of Interest: The authors state that there is no conflict of interest.

References

1. Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR:towards an international standard. Appl Environ Microbiol. 2003;69(1):290-296. DOI: 10.1128/AEM.69.1.290-296.2003
2. Dróżdż M, Małaszczuk M, Paluch E, Pawlak A. Zoonotic potential and prevalence of *Salmonella* serovars isolated from pets. Infect Ecol

Epidemiol. 2021;11(1):197-530. DOI: 10.1080/20008686.2021.1975530
3. Lowden P, Wallis C, Gee N, Investigating the prevalence of *Salmonella* in dogs within the Midlands region of the UK. BMC Vet Res. 2015;11(1):2-8. DOI: 10.1186/s12917-015-0553-z
4. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Salmonellosis harrison's principles of internal medicine. 17th ed. New York: McGraw-Hill. 2008; 956-961 p. DOI: 10.1111/j.1445-994.2008.01837.x
5. Jesny B, Sabri II, Al-Sultan K, Sherly P, Mohammed JS. Pathogenesis of *Salmonella enterica* serovar *albany* in experimental infected SPF BALB/c Mice. Iraqi J Vet Sci. 2020;34(2):339-344. DOI: 10.33899/ijvs.2019.126269.1282
6. Fàbrega A, Vila J. *Salmonella enterica* serovar *Typhimurium* skills to succeed in the host: Virulence and regulation. Clin Microbiol Rev. 2013;26(2):308-41. DOI: 10.1128/CMR.00066-12
7. Dieckmann R, Malorny B. Rapid screening of epidemiologically important *Salmonella enterica subsp. enterica* serovars by whole-cell matrix-assisted laser desorption ionization- time of flight mass spectrometry. Appl Environ Microbiol. 2011;77(12),4136-4146. DOI: 10.1128/AEM.02418-10
8. Weigel RM, Qiao B, Teferedegne B, Suh DK, Barber DA, Isaacson RE, White BA. Comparison of pulsed-field gel electrophoresis and repetitive sequence polymerase chain reaction as genotyping methods for detection of genetic diversity and inferring transmission of *Salmonella*. Vet Microbiol. 2004;100(3):205-217. DOI: 10.1016/j.vetmic.2004.02.009
9. Andrews-Polymenis HL, Santiviago CA, McClelland M. Novel genetic tools for studying food-borne *Salmonella*. Curr Opin Biotechnol. 2009;20(2):149-157. DOI: 10.1016/j.copbio.2009.02.002
10. Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA. Worldwide Epidemiology of *Salmonella* Serovars in animal- based foods:a meta- analysis. Appl Environ Microbiol. 2019;85(14):00591-19. DOI: 10.1128/A EM.00591-19
11. Alkhafaje WK, Olama ZA, Ali SM. Molecular characterization and microbial resistance of different bacterial isolates in some dairy products. Iraqi J Vet Sci. 2022;36(2):333-339. DOI: 10.33899/ijvs.2021.13 0206.1764
12. Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, Helmuth R, Hauser E, Guerra B, Beutlich J, Brisabois A, Peters T, Svensson L, Madajczak G, Litrup E, Imre A, Herrera-Leon S, Mevius D, Newell DG, and Malorny B. Virulotyping and antimicrobial resistance typing of *Salmonella enterica* serovars relevant to human health in Europe. Foodborne Pathog. Dis. 2009;7:523-535. DOI: 10.1089/fpd.2009.0447
13. Haneda T, Okada N, Nakazawa N, Kawakami T, Danbara H. Complete DNA sequence and comparative analysis of the 50-Kilobase virulence plasmid of *Salmonella enterica* serovar *Cholerasuis*. Infect Immun. 2001;69:2612-2620. DOI: 10.1128/IAI.69.4.2612-2620.2001
14. Case RJ, Boucher Y, Dahllöf I, Holmström C, Doolittle WF, Kjelleberg S. Use of *16S rRNA* and *rpoB* genes as molecular markers for microbial ecology studies. Appl Environ Microbiol. 2007;73(1):278-88. DOI: 10.1128/AEM.01177-06
15. Bosshard PP, Zbinden R, Abels S, Böddinghaus B, Altwegg M, Böttger EC. *16S rRNA* gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of nonterminating Gram-negative bacteria in the clinical laboratory. J Clin Microbiol. 2006;44(4):1359-66. DOI: 10.1128/JCM.44.4.1359-1366.2006
16. Lu JJ, Perng CL, Lee SY, Wan CC. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J Clin Microbiol. 2000;38:2076-2080. DOI: 10.1128/JCM.38.6.2076-2080.2000
17. Baker GC, Smith JJ, Cowan DA. Review and re-analysis of domain-specific 16SrRNA primers. J Microbiol Meth. 2003;55(1):541-555. DOI: 10.10 16/j.mimet.2003.08.009
18. Clarridge JE. Impact of *16S rRNA* gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinl Microbiol Rev. 2004;17(1):840-1862. DOI: 10.1128/CMR.17.4.840-862.2004
19. Saeed ZK, Abbas BA, Othman RM. Molecular identification and phylogenetic analysis of *lactic acid* bacteria isolated from goat raw milk. Iraqi J Vet Sci. 2020;34(2):259-263. DOI: 10.33899/ijvs.125896.1176
20. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of *16S ribosomal RNA* gene segments for the diagnosis of pathogenic bacteria. J Microbiol Meth. 2007;69(2):330-9. DOI: 10.1016/j.mimet.2007.02.005
21. Masek J, Hardick J, Won H, Yang S, Hsieh Y, Rothman R, Gaydos C. Sensitive detection and serovar differentiation of typhoidal and nontyphoidal *Salmonella enteric* species using *16S rRNA* Gene PCR coupled with high-resolution melt analysis. J Med Diag. 2013;16(2):261-266. DOI: 10. 1016/j.jmoldx.2013.10.011
22. Jeng K, Yang S, Won H, Gaydos CA, Hsieh YH, Kecojevic A, Carroll KC, Hardick J, Rothman RE. Application of a 16S rRNA PCR- high-resolution melt analysis assay for rapid detection of *Salmonella* Bacteremia. J Clin Microbiol. 2012;50(3):1122-1124. DOI: 10.1128/JCM.05121-11
23. Maser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Sze H, Talke IN, Amtmann A, Maathuis FJ. Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol. 2001;126(1):1646-1667. DOI: 10.1104/pp.126.4.1646
24. Edwards SV. Is a new and general theory of molecular systematics emerging. Evolut. 2009;63(1):1-19. DOI: 10.1111/j.1558-5646.2008.00549.x
25. Grenfell BT, Pybus OG, Gog JR, Wood JL, Daly JM, Mumford JA, Holmes EC. Unifying the epidemiological and evolutionary dynamics of pathogens. Sci. 2004;303:327-332. DOI: 10.1126/science.1090727
26. Brady A, Salzberg S, Phymm BL. expanded: Confidence scores, custom databases, parallelization, and more. Nat Meth. 2011;8(1):511-367. DOI: 10.1038/nmeth0511-367
27. Ye SH, Siddle KJ, Park DJ, Sabeti PC. Benchmarking metagenomics tools for taxonomic classification. Cell. 2019;178(4):779-794. DOI: 10.1016/j.cell.2019.07.010
28. Li H, Durbin R. Inference of human population history from individual whole-genome sequences. Nat. 2011;475:493-496. DOI: 10.1038/nature10231
29. Ma J. Reconstructing the history of large-scale genomic changes: Biological questions and computational challenges. J Comput Biol. 2011;18(1):879-893. DOI: 10.1089/CMB.2010.0189
30. Sharif YM, Tayeb BA. Estimation of limit of detection of *Salmonella typhimurium* in artificially contaminated chicken meat by cultured-based and polymerase chain reaction techniques. Iraqi J Vet Sci. 2021;35(4):621-625. DOI: 10.33899/ijvs.2020.127328.1496
31. Nyabundi D, Onkoba N, Kimathi R, Nyachieo A, Juma G, Kinyanjui P, Kamau J. Molecular characterization and antibiotic resistance profiles of *Salmonella* isolated from fecal matter of domestic animals and animal products in Nairobi. Trop Dis Travel Med Vaccines. 2017;3(2):1-4. DOI: 10.1186/s40794-016-0045-6
32. James HJ, Karen CC, Guido F, Michael AP, Marie LL, Sandra S R, David WW. Manual Clin Microbiol. 2015;11(1):702. DOI: 10.1128/9781555817381
33. Sambrook J, Perng CL, Lee SY, Wan CC. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. J Clin Microbiol. 2000;38:2076-2080. DOI: 10.1128/JCM.38.6.2076-2080.2000
34. Kumar S, Stecher G, Li M, Knyaz C, Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms.

الشجرة العرقية لسلالات السالمونيلا المعوية للنوع التحت المعوي المعزولة من الإنسان والحيوان في محافظتي البصرة وبغداد، العراق

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الخلاصة

تعد القرابة الوراثية للأنماط المصلية للسالمونيلا المعوية للنوع التحت المعوي المعزولة من الإنسان والحيوان ذات أهمية لان معلوماتها توفر دليلا مهماً لتحديد مصدر العدوى البشرية. لذا هدفت الدراسة الحالية إلى تحديد العلاقة الوراثية للسالمونيلا المعوية تحت النوع المعوي المعزولة من الإنسان والحيوان. جمعت ٣٠٠ عينة من مصدرين رئيسيين بشري وحيواني في محافظتي البصرة وبغداد. لبناء الشجرة الوراثية لجراثيم السالمونيلا المعوية تحت النوع المعوي تم تحليل نتائج تحديد التتابعات الجينات *16r RNA* و*spvC* و*avrA* باستخدام تحليل بلاست لمطابقة النتائج مع البنك الجيني العالمي ولمعرفة نسبة التشابه والاختلاف بين السلالات العراقية والعالمية، تبين أن معدل التشابه في الجين الأول ٩٧,٧٧% والجين الثاني ٩٨,٢٩% والثالث ٩٦,٨٢% على التوالي. تم إنشاء الشجرات الوراثية لكل جين من الجينات الثلاث على حدي باستخدام طريقتين الأولى: الإمكانية الأكثر للتشابه والثانية اقل تطور موجود مع إضافة مجموعة من السلالات الأجنبية المسجلة في البنك الجيني العالمي لمعرفة موقع السلالة العراقية منها ، وتم إيجاد ترابط بين السالمونيلا العراقية والعالمية في الجين الأول *16sr RNA*ولكن أظهرت النتائج اختلاف في تسلسل بعض قواعد لجيني *avrA* و *spvC*، إن التشابه الكبير بين تسلسل قواعد *16sr RNA* مع السلالات الأجنبية وهذا يقودنا للاستنتاج بأن السالمونيلا العراقية تميزت بمسارها الخاص في تطوير سلالات عالمية مع بعض الارتباط في بعض العينات أو وجود صلة مع نفس الأجداد التي نهضت منه السالمونيلا المشخصة في العراق.

Mol Biol Evolut. 2018;35(6):1547-1549. DOI: 10.1093/molbev/msy096

35. Ranjbar R, Ahmadi M and Memariani M. Multiple-locus variable-number tandem repeat analysis (MLVA) for genotyping of *Salmonella Enterica subspecies enterica* serotype infantis isolated from human sources. Microb Pathog. 2016;100(1):299-304. DOI: 10.1016/j.micpath.2016.10.012
36. Fricke WF, Mammel MK, McDermott PF, Tartera C, White DG, Le Clerc JE, Cebula TA. Comparative genomics of 28 *Salmonella Enterica* isolates: Evidence for CRISPR-mediated adaptive sublineage evolution. J Bacteriol. 2011;193(14):3556-68. DOI: 10.1128/JB.00297-11
37. Mahapatro G, Mishra D, Shaw K, Mishra S, and Jena T. Phylogenetic tree construction for DNA sequences using clustering methods. Proced Eng. 2012;38(1):1362-1366. DOI: 10.1016/j.proeng.2012.06.169
38. Mignard S, Flandrois JP. *16S rRNA* sequencing in routine bacterial identification: A 30-month experiment. J Micro Biol Meth. 2006;67(1):574-581. DOI: 10.1016/j .mimet.2006.05.009
39. Gregory TR. Understanding Evolutionary Trees. Evol Edu Outreach. 2008;1(1):121-137. DOI: 10.1007/s12052-008-0035-x
40. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob Resist Infect Control. 2017;6(1):47. DOI: 10.1186/s13756-017-0208-x
41. Liu H, Whitehouse CA, Li B. Presence and Persistence of *Salmonella* in Water: the impact on microbial quality of water and food safety. Front Public Hlth. 2018;6(1):159. DOI: 10.3389/fpubh.2018.00159